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How Sugar Tunes Your Clock

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While cellular circadian clocks are set by the light/dark cycle, these clocks can be reset by what we eat. Two papers in this issue of *Cell Metabolism* reveal that *O*-GlcNAcylation of clock proteins, which is dependent on nutrients, adjusts our circadian clock (Kaasik et al., 2013; Li et al., 2013).

All of our cells have an ~24 hr clock (termed the circadian clock) that is primarily set by light but is also modified by environmental cues, especially nutrients, such as glucose (Damiola et al., 2000). However, the molecular mechanism of nutrient regulation of our cellular clocks is largely unknown. Current models suggest that our circadian clocks are comprised of transcriptional autoregulatory feedback loops controlled by specific transcription factors (Mohawk et al., 2012). Prior work has shown that the rate of cycling of the clock autofeedback loops are modulated by several regulatory mechanisms including post-translational modifications (PTMs) of clock transcription factors by acetylation, ADP-ribosylation, phosphorylation, and ubiquitination (Asher and Schibler, 2011). For example, in one such autofeedback loop, heterodimers of the transcription factors CLOCK and BMAL1 bind E-box elements in DNA to activate the expression of Period (Per) and Chryptochrome (Cry) proteins, which accumulate in the cytosol until they are phosphorylated and enter the nucleus where they inhibit the activity of CLOCK/BMAL1 (Figure 1). Degradation of the inhibitory Per and Cry proteins restarts a new cycle of the circadian clock (Ukai and Ueda, 2010). These transcription factor feedback loops are highly conserved in biology.

O-GlcNAcylation (Hart et al., 2011; Hart et al., 2007) is the covalent attachment and cycling of N-acetylglucosamine (GlcNAc) moieties on serine or threonine residues of nucleocytoplasmic proteins. Intracellular concentrations of UDP-GlcNAc, the donor for *O*-GlcNAcylation, are controlled by flux through several major metabolic pathways including metabolism of glucose, amino acids, and fatty acids. Because *O*-GlcNAcylation is highly responsive to UDP-GlcNAc concentrations, *O*-GlcNAc serves as a major metabolic/nutrient sensor (Hart et al., 2011). Prior work by Durgan et al. (2011) in mouse hearts showed that a diurnal cycle of *O*-GlcNAcylation is controlled by circadian clock regulation of glucose uptake, glutamine synthesis, and *O*-GlcNAc transferase (OGT) and *O*-GlcNAcase (OGA) protein levels, which add and remove *O*-GlcNAc from target substrates, respectively. They also showed that *O*-GlcNAcylation of the clock transcription factor Bmal1 reduced Per2 protein levels and phase-advanced the clock. These findings suggested that the circadian clock increases *O*-GlcNAcylation during the active/wake phase and that the sugar modification in turn modulates the timing of the circadian clock. Kim et al. (2012) further showed in *Drosophila* that *O*-GlcNAcylation of the Per transcription factor displays a circadian cycle, which tunes the clock by controlling Per nuclear entry and stabilization. Two papers published in this issue of *Cell Metabolism* (Kaasik et al., 2013; Li et al., 2013) have now substantially advanced our understanding of nutrient regulation of circadian clocks by showing that protein *O*-GlcNAcylation fine-tunes and adjusts our cellular clocks via the sugar's interplay with both phosphorylation and ubiquitination of clock regulatory proteins.

Using an unbiased phosphoproteomics approach, Kaasik et al. (2013) show that circadian cycling of phosphorylation regulates GSK3β kinase-dependent

signaling pathways in mouse hippocampus and liver, which in turn regulate many key metabolic pathways (e.g., insulin signaling). Their analyses also reveal that OGT is phosphorylated by GSK3β, increasing the glycosyltransferase's activity, and that the two enzymes exhibit reciprocal regulation (Figure 1). Using primary embryonic fibroblasts from *Per2-luciferase* transgenic mice, in combination with OGT or OGA inhibitors, Kaasik et al. (2013) found that global alterations in *O*-GlcNAcylation alters the circadian period length in mice. Similar results were found using RNAi knockdowns of the *O*-GlcNAc cycling enzymes in *Drosophila*. Increased *O*-GlcNAcylation, as occurs when nutrients are in excess, lengthens the circadian rhythmicity, and decreased *O*-GlcNAcylation shortens the circadian rhythmicity. *O*-GlcNAcylation of CLOCK and Per, key transcription factors controlling the circadian clock, regulates their transcriptional activities, with *O*-GlcNAcylation repressing CLOCK activation of Period. Furthermore, glucose-regulated *O*-GlcNAcylation of PER2 competes with phosphorylation events in a region of the protein known to regulate human sleep. The authors suggest that *O*-GlcNAcylation of PER2 likely fine-tunes clock speed in response to nutrients.

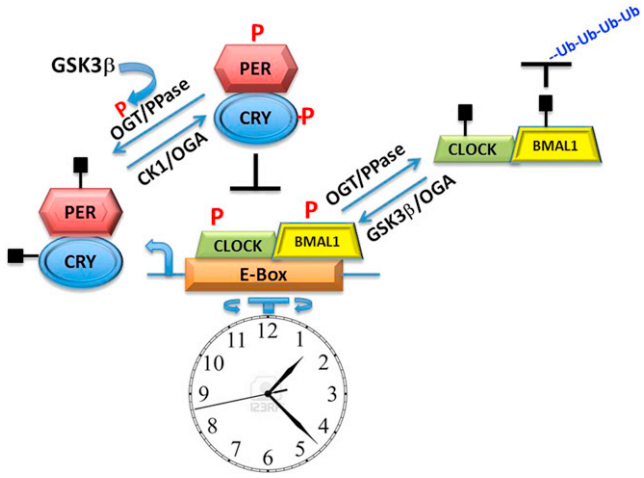

Figure 1. Reciprocal Modification of Circadian Clock Proteins by *O*-GlcNAcylation and Phosphorylation Tunes the Circadian Clock in Response to Nutrients

Circadian clocks are comprised of transcriptional autoregulatory feedback loops controlled by specific transcription factors. A circadian timing circuit is shown in which the transcription factors CLOCK and BMAL1 activate the transcription of PER and CRY. PER/CRY accumulate in the cytosol until they become phosphorylated and enter the nucleus where they repress their induction by CLOCK/BMAL1. Degradation of PER/CRY resets the cycle. Kaasik et al. (2013) found that GSK3β activity cycles with the circadian clock, and phosphorylation of *O*-GlcNAc transferase (OGT) by GSK3β activates the enzyme. Activated OGT in turn glycosylates the CLOCK, PER, and CRY transcription factors, preventing their phosphorylation. *O*-GlcNAcylation of CLOCK represses activation of PER, and *O*-GlcNAcylation of PER competes with phosphorylation of PER at sites known to regulate human sleep. *O*-GlcNAcylation of PER fine-tunes clock speed in response to nutrients. Li et al. (2013) show that *O*-GlcNAcylation and phosphorylation also crosstalk competitively to regulate the ubiquitin-mediated degradation of the CLOCK and BMAL1 circadian clock transcription factors. Black square, *O*-GlcNAc; P, phosphate; Ub, ubiquitin; OGT, *O*-GlcNAc transferase; OGA, *O*-GlcNAcase; CK1, CK1 kinase; PPase, protein phosphatase; GSK3β, glycogen synthase kinase 3 beta.

Li et al. (2013) also report that *O*-GlcNAcylation regulates the circadian clock, but by a different mechanism, and they provide further support for the hypothesis that glucose availability regulates the circadian clock in the liver via flux through the hexosamine/*O*-GlcNAc pathway. They show that glucose-dependent *O*-GlcNAcylation and ubiquitination work in concert to reciprocally regulate or tune the circadian clock (Figure 1). Circadian rhythmic *O*-GlcNAcylation, as catalyzed by OGT, entrains the transcription of BMAL1/CLOCK target gene expression by blocking ubiquitin-mediated degradation of the BMAL1 and CLOCK proteins. Genetically increasing or decreasing OGT expression in the liver of mice induces aberrant circadian rhythms in glucose homeostasis: overexpression of OGT increases the diurnal rhythm of blood glucose, and reduced OGT expression advances the rhythm of circulating glucose by 6–8 hr, contributing to hyperglycemia in the daytime.

Collectively, the studies by Kaasik et al. (2013) and Li et al. (2013) indicate that *O*-GlcNAcylation, which is a major nutrient sensor, tunes our circadian clocks not only by regulating the dynamic phosphorylation of transcription factors comprising the timing circuits, but also by controlling the turnover of the clock proteins by blocking their degradation via the ubiquitin/proteasome pathway. Logic suggests that our circadian clocks are critical for adapting our energy metabolism to meet our needs during the sleep/wake cycle. These studies reveal important fundamental mechanisms of how nutrient intake and eating habits modulate our circadian metabolism and cellular functions in a coordinate manner. They also illustrate that cellular metabolism and signaling are much more complex and highly regulated than our current phosphorylation-centric models suggest. A molecular understanding of how nutrients tune our circadian clocks will be critically important toward elucidation of how dysregulation of these clocks contributes to chronic diseases such as obesity and diabetes.

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